UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EPA United States Environmental Protection Agency **Office of Pesticide Programs**

July 21, 2011

MEMORANDUM

SUBJECT: Acute Toxicity Review for File Symbol: 777-RRT#
Product Name: Edelweiss-Trigger
DP Barcode: 390042

FROM: Earl Goad, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

THRU: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

TO: Marshall Swindell PM#33/Martha Terry
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, LLC.

PRODUCT FORMULATION FROM LABEL:

PC Codes	Active Ingredient(s):	% by wt.
000595	Hydrogen Peroxide	0.88
	Other Ingredient(s):	99.12
	Total:	100.00

I) BACKGROUND:

Reckitt Benckiser has submitted an application for registration of their new residential and commercial end-use cleaner/disinfectant for use on hard, non-porous surfaces.

This acute toxicity package consisted of the following documents:

1. Transmittal Document/ Letter from the registrant dated May 4, 2011.
2. Proposed product label dated May 4, 2011.
3. Five Confidential Statements of Formula dated May 4, 2011. Basic CSF, three alternate CSFs identified by different fragrances, and one alternate which is fragrance free.
4. A Chemical Characterization for Toxicological Study of Formula 1563-125A (Basic), MRID# 484737-07
5. Six pack of original studies which include: Acute Oral (MRID 484737-10), Acute Dermal (MRID 484737-11), Acute Inhalation (MRID 484737-12), Primary Eye Irritation (MRID 484737-13), Primary Skin Irritation (MRID 484737-14), and Dermal Sensitization (MRID 484737-15).

Since the final product name was not yet finalized at the initiation of these studies, the registrant reports that these Acute Toxicity studies were performed using the subject product designated as Edelweiss BRC.

A primary review of these original studies was conducted by the Product Science Branch (PSB)/Antimicrobials Division (AD) contractor: Computer Sciences Corporation (CSC). The Chemistry and Toxicology Team (CTT) conducted a brief secondary review to assure that the studies, any citations or data waivers meet EPA/OPP criteria, and is responsible for this memorandum.

II) FINDINGS: PSB findings are:

A. The applicant's letter to EPA (dated May 4, 2011) states that the product Edelweiss BRC and Formula # 1563-125A, are identical and synonymous in any combination with the product name, Edelweiss - Trigger, which is the product for which registration is sought.

B. The study identified as "A Chemical Characterization for Toxicological Study of Formula 1563-125A (Basic), MRID# 484737-07 confirmed that the concentration of the active ingredient (Hydrogen peroxide) in the product used for these Acute Toxicity studies is consistent with its labeled concentration. The concentration of Hydrogen peroxide was within the required certified limits at the initiation of these Acute Toxicity studies and at their completion.

	% weight Hydrogen peroxide
Label (Nominal) concentration Hydrogen peroxide	0.88%
Initial Assay value December 15, 2009	1.09
Final Assay value June 21, 2010	1.06

C. The Acute Oral Toxicity study was performed using the Up and Down procedure. All of the rats which were dosed at 5,000 mg/kg died. The animals dosed at the alternate dose of 1750 mg/kg survived. These results are determined to be category IV and are found to be acceptable.

D. Acute Dermal Toxicity study. The rats were dosed with a limit dose of 5,000 mg/kg, all animals survived. Two of the five females showed some erythema for the first few days. These results are determined to be category IV and are found to be acceptable.

E. Acute Inhalation Toxicity study. The rats were dosed (nose-only) with a limit dose of 2.06 mg/L. All animals survived and were observed to be active and healthy. These results are determined to be category IV and are found to be acceptable.

F. Primary Eye Irritation study. All 3 rabbits showed slight conjunctival irritation, no signs of irritation to cornea or iris were observed. These results are determined to be category IV and are found to be acceptable.

G. Primary Skin Irritation study. All observed signs of skin irritation (erythema or edema) cleared by 72 hours. These results are determined to be category IV and are found to be acceptable.

H. Dermal Sensitization (Buehler Method). The testing material was found not to be a sensitizer. This result is found to be acceptable.

III) The acute toxicity profile for EPA File Symbol: 777-RRT "Edelweiss -Trigger" is currently:

Study	MRID Number	Toxicity Category	Status
Acute Oral Toxicity	484737-10	IV	Acceptable
Acute Dermal Toxicity	484737-11	IV	Acceptable
Acute Inhalation Toxicity	484737-12	IV	Acceptable
Primary Eye Irritation	484737-13	IV	Acceptable
Primary Skin Irritation	484737-14	IV	Acceptable
Dermal Sensitization	484737-15	Non-Sensitizer	Acceptable

IV) LABELING:

A. Child Hazard Warning Statement is required.

Keep Out of Reach of Children

B. A signal word for EPA File Symbol Edelweiss – Trigger is not required because the all of the Acute Toxicity studies resulted in category IV except in the case of Dermal Sensitization a Non-sensitizer.

C. Precautionary labeling: No additional precautionary labeling is required, except if the registrant chooses to do so, and then the signal word of "Caution" and category III labeling may be assumed.

D. Based on the Acute Toxicity profile, first aid statements are required unless the registrant chooses include them. In the situation where the registrant chooses to use additional precautionary statements, the appropriate First Aid Statements for the exposure route must be listed

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)

Product Manager: 33
MRID No.: 484737-10

Reviewer: Earl Goad
Completion Date: May 11, 2010
Study No.: 28907

Testing Laboratory: Eurofins | PSL, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), 1989.

Test Material: Edelweiss-BRC, Formula # 1563-125A
Batch #: 1621-053 / Colorless liquid

Dosage: Limit Test: 5,000 mg/kg (administered as received)

Species: 3 Rats; Sprague-Dawley derived, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (9 weeks old)
Weight: 170-178 grams at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 19-22°C
Humidity Range: 30-58%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 6-7 days

Conclusion:

1. **Acute Oral LD_{50} (mg/kg)**: Female Rats: >5,000 mg/kg

2. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1100): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The guidelines state that the animals are to be observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours, and daily thereafter. The animals were observed during the first several hours post-dosing, and at least once daily thereafter for 14 days after dosing.
- The guidelines state that body weight changes should be calculated and recorded. Individual body weights of test animals were recorded; however, body weight changes were not reported.
- The laboratory report describes a study conducted for the product, Edelweiss-BRC Formula # 1563-125A. The applicant's letter to EPA (dated May 4, 2011) states that the product names, Edelweiss BRC and Formula # 1563-125A, are identical and synonymous in any combination with the product name, Edelweiss - Trigger, which is the product for which registration is sought.

Results:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	5,000	S	S
2	3102	5,000	S	S
3	3103	5,000	S	S

S – Survival

Observations:
All animals survived, gained body weight, and appeared active and healthy during the study. No signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior were noted.

Gross Necropsy Findings:
No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 33 **Reviewer**: Earl Goad
MRID No.: 484737-11 **Completion Date**: May 11, 2010
Study No.: 28908

Testing Laboratory: Eurofins | PSL, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12). A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), 1989.

Test **Material**: Edelweiss-BRC, Formula # 1563-125A
Batch #: 1621-053 / Colorless liquid

Dosage: 5,000 mg/kg (applied as received)

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8-9 weeks old)
Weight: Males: 242-279 grams; Females: 177-202 grams; at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 20-23°C
Humidity Range: 46-59%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 9 days

Summary:

1. **Acute Dermal LD_{50} (mg/kg)**:
   Male and Female Rats: >5,000 mg/kg

2. **The estimated acute dermal LD_{50} is** greater than 5,000 mg/kg in male and female rats.

3. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1200): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The guidelines state that body weight changes should be calculated and recorded when survival exceeds one day. Individual body weights of test animals were recorded; however, body weight changes were not reported.
- The laboratory report describes a study conducted for the product, Edelweiss-BRC Formula # 1563-125A. The applicant's letter to EPA (dated May 4, 2011) states that the product names, Edelweiss BRC and Formula # 1563-125A, are identical and synonymous in any combination with the product name, Edelweiss - Trigger, which is the product for which registration is sought.

Results:

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
5,000	0 / 5	0 / 5	0 / 10

Observations:
All animals survived exposure to the test substance and gained body weight during the study. Other than the dermal irritation (erythema) noted at two dose sites between Days 1 and 2, no other clinical findings were recorded for any animal over the course of the study.

Gross Necropsy Findings:
No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)
(NOSE-ONLY EXPOSURE)

Product Manager: 33
MRID No.: 484737-12

Reviewer: Earl Goad
Completion Date: May 11, 2010
Study No.: 28909

Testing Laboratory: Eurofins | PSL, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), 1989.

Test Material: Edelweiss-BRC, Formula # 1563-125A
Batch #: 1621-053 / Colorless liquid

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8-9 weeks old)
Weight: Males: 240-250 grams; Females: 181-204 grams; at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 20-23°C
Humidity Range: 40-62%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 7 days

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.06	116.57

Summary:

1. **LC_{50} (mg/L) 4-hr exposure:**

   >2.06 mg/L in male and female rats

2. **The estimated 4-hr acute inhalation LC_{50} of Edelweiss-BRC is greater than 2.06 mg/L in male and female rats.**

3. **Average MMAD:** 1.6 µm

4. **Toxicity Category:** IV

   Classification: Acceptable

Procedure (Deviations from 870.1300): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following protocol amendment: "A clarification was done on the Protocol Section: 8.E.1 Gravimetric Concentration ... TO: Gravimetric concentration will be determined by using 37 mm glass fiber filters (GF/B Whatman or equivalent) in filter holders attached by Tygon tubing to an electric vacuum pump."
- The guidelines state that the animals should be acclimated and heat stressed minimized. The laboratory did not indicate whether animals were acclimated to exposure conditions and heat stress minimized.
- The guidelines state that three to four measurements should be taken during exposure if chamber concentrations and MMAD values taken during the trial run measurements are not within 10 percent of each other. Chamber concentrations ranged from 0.23 to 2.05 mg/L during the pre-test trials. MMAD values were reported for one of the three trial runs. The laboratory conducted only two sample measurements during the test, instead of the three to four measurements recommended in the guidelines.
- The guidelines state that body weight changes should be calculated and recorded when survival exceeds 1 day. Individual body weights of test animals were recorded; however, body weight changes were not reported.
- The laboratory report describes a study conducted for the product, Edelweiss-BRC Formula # 1563-125A. The applicant's letter to EPA (dated May 4, 2011) states that the product names, Edelweiss BRC and Formula # 1563-125A, are identical and synonymous in any combination with the product name, Edelweiss - Trigger, which is the product for which registration is sought.

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number Dead / Number Tested		
	Males	Females	Combined
2.06	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exp. Conc. (mg/L)	Sample	MMAD (µm)	GSD (µm)	Cumulative % of Particles < Effective Cutoff Diameter (µm)[1]								
				0.0	0.4	0.7	1.1	2.1	3.3	4.7	5.8	9.0
2.06	1	1.6	2.60	0.0	6.8	18.8	35.1	62.5	75.4	86.8	91.4	97.2
	2	1.6	2.57	0.0	6.7	18.2	34.7	63.5	76.5	86.3	90.9	96.5

[1]Percent of particles smaller than corresponding effective cutoff diameter

Chamber Environment During Exposure

Exposure Level (mg/L)	2.06
Chamber Volume (L)	~6.7
Average Total Airflow Volume (Lpm)[1]	25.7
Air Changes Per Hour	230
Mean Oxygen Content (%)	not reported
Temperature Range (°C)	21-23
Relative Humidity Range (%)	40-46

[1]Total air = filtered, compressed air + compressed mixing air

Clinical Observations:
All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. Following exposure and throughout the 14-day observation period, all animals appeared active and healthy. No signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior were noted.

Gross Necropsy Findings:
No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 33
MRID No.: 484737-13

Reviewer: Earl Goad
Completion Date: May 11, 2010
Study No.: 28910

Testing Laboratory: Eurofins | PSL, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), 1989.

Test Material: Edelweiss-BRC, Formula # 1563-125A
Batch #: 1621-053 / Colorless liquid

Dosage: 0.1 mL (instilled as received)

Species: 3 Rabbits; New Zealand, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (specific age not provided)
Weight: Information not provided (and not required)
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature Range: 19-21°C
Humidity Range: 18-57%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 26-33 days

Summary:

1. **Toxicity Category**: IV

2. **Classification**: Acceptable

Procedure (Deviations from 870.2400): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- The laboratory reported the following: "The humidity was below the targeted lower limit for 1 day during the study. A portable humidifier was used to raise the humidity levels during this time."
- The guidelines recommend that testing be performed using healthy adult albino rabbits. Testing was performed using young adult albino rabbits (specific age not provided).

- The laboratory report describes a study conducted for the product, Edelweiss-BRC Formula # 1563-125A. The applicant's letter to EPA (dated May 4, 2011) states that the product names; Edelweiss BRC and Formula # 1563-125A. are identical and synonymous in any combination with the product name, Edelweiss - Trigger, which is the product for which registration is sought.

Results:
All animals appeared active and healthy during the study. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

No corneal opacity or iritis was noted for any treated eye during the study. One hour after test substance instillation, all three treated eyes exhibited conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 72 hours (study termination). The Maximum Mean Total Score of Edelweiss-BRC is 6.0. Under the conditions of this study, Edelweiss-BRC is classified as mildly irritating to the eye.

Incidence of Irritation

Time Post Instillation	No. of Animals Testing "Positive" / No. of Animals Tested			Severity – Mean Score
	Corneal Opacity	Iritis	Conjunctivae	
1 hour	0 / 3	0 / 3	1 / 3	6.0
24 hours	0 / 3	0 / 3	0 / 3	2.0
48 hours	0 / 3	0 / 3	0 / 3	0.7
72 hours	0 / 3	0 / 3	0 / 3	0.0

Individual Scores for Ocular Irritation

Observations	Rabbit No. 3401 (Female)				Rabbit No. 3402 (Female)				Rabbit No. 3403 (Female)			
	Hours After Treatment											
	1	24	48	72	1	24	48	72	1	24	48	72
I. Corneal Opacity	0	0[1]	0	0	0	0[1]	0	0	0	0[1]	0	0
II. Iritis	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	1	0	0	0	2	1	1	0	1	1	0	0
B. Chemosis	0	0	0	0	1	0	0	0	1	0	0	0
C. Discharge	1	0	0	0	1	1	0	0	1	0	0	0

[1]2% ophthalmic fluorescein sodium used to verify the absence of corneal opacity

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING
(OPPTS 870.2500)

Product Manager: 33
MRID No.: 484737-14

Reviewer: Earl Goad
Completion Date: May 11, 2010
Study No.: 28911

Testing Laboratory: Eurofins | PSL, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), 1989.

Test Material: Edelweiss-BRC, Formula # 1563-125A
Batch #: 1621-053 / Colorless liquid
Dosage: 0.5 mL (applied as received)
Species: 3 Rabbits; New Zealand, albino
Sex: Males
Age: Young adult (specific age not provided)
Weight: Information not provided (and not required)
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature Range: 20-22°C
Humidity Range: 31-35%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 6 days

Summary:

1. **Toxicity Category:** IV

2. **Classification:** Acceptable

Procedure (Deviations from 870.2500): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The guidelines recommend that testing be performed using healthy adult animals. Testing was performed using young adult animals (specific age not provided).
- The laboratory report describes a study conducted for the product, Edelweiss-BRC Formula # 1563-125A. The applicant's letter to EPA (dated May 4, 2011) states that the product names, Edelweiss BRC and Formula # 1563-125A, are identical and synonymous in any combination with the product name, Edelweiss - Trigger, which is the product for which registration is sought.

Results:
All animals appeared active and healthy during the study. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

One hour after patch removal, all three treated sites exhibited well-defined erythema and slight edema. The overall reported incidence and severity of irritation decreased with time. All animals were free of dermal irritation by 72 hours.(Note: In the summary remarks it was stated that "The overall incidence and severity of irritation increased with time" – this is found to be incorrect considering after evaluating the data, which proves otherwise.

The Primary Dermal Irritation Index for Edelweiss-BRC was calculated to be 1.8. [Scores for observations made during the first 30-60 minutes, 24, 48, and 72 hours were used in this calculation.] Under the conditions of this study, Edelweiss-BRC is classified as slightly irritating to the skin.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
30-60 minutes	3 / 3	3 / 3
24 hours	3 / 3	3 / 3
48 hours	1 / 3	0 / 3
72 hours	0 / 3	0 / 3

Individual Skin Irritation Scores

Animal No.	Sex	Erythema / Edema			
		Time After Patch Removal			
		30-60 minutes	24 hours	48 hours	72 hours
3501	M	2 / 2	2 / 1	0 / 0	0 / 0
3502	M	2 / 2	2 / 1	0 / 0	0 / 0
3503	M	2 / 2	2 / 1	1 / 0	0 / 0
Total		6 / 6	6 / 3	1 / 0	0 / 0
Mean		2.0 / 2.0	2.0 / 1.0	0.3 / 0.0	0.0 / 0.0

Summary of Skin Irritation Scores[1]

	Time After Patch Removal			
	30-60 minutes	24 hours	48 hours	72 hours
Erythema	2.0	2.0	0.3	0.0
Edema	2.0	1.0	0.0	0.0
TOTAL (PDI)[2]	4.0	3.0	0.3	0.0

[1]Average values for three rabbits
[2]PDI = Average Erythema + Average Edema

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(BUEHLER METHOD)

Product Manager: 33
MRID No.: 484737-15

Reviewer: Earl Goad
Completion Date: May 11, 2010
Study No.: 28912

Testing Laboratory: Eurofins | PSL, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), 1989, with the following exception: "The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during Eurofins PSL historical positive control study were not determined."

Test Material: Edelweiss-BRC, Formula # 1563-125A
Batch #: 1621-053 / Colorless liquid

Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA)
Historical validation study – Completed on December 30, 2009

Species: 34 Guinea pigs; Hartley, albino
Sex: Range-Finding: 3 Males and 1 Female
Test Group: 20 Males
Naïve Control Group: 10 Males
Female was nulliparous and non-pregnant.
Age: Young adult (specific age not reported)
Weight: Test and Naïve Control Groups: 318-410 grams at experimental start
Source: Elm Hill Breeding Labs, Chelmsford, MA
Housing: Temperature Range: 19-22°C
Humidity Range: 39-59%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 7-34 days

Method: Buehler Method

Summary:

1. Based on these findings and on the evaluation system used, Edelweiss-BRC is not considered to be a contact sensitizer.

2. Classification: Acceptable

Procedure (Deviations from 870.2600): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The guidelines state that, as a minimum, the erythema and edema must be graded. The laboratory only graded erythema.
- The guidelines state that the number of animals per cage is to be reported. The laboratory reported that "animals were group housed in suspended stainless steel caging." The laboratory did not report the number of animals per cage.
- The laboratory report describes a study conducted for the product, Edelweiss-BRC Formula # 1563-125A. The applicant's letter to EPA (dated May 4, 2011) states that the product names, Edelweiss BRC and Formula # 1563-125A, are identical and synonymous in any combination with the product name, Edelweiss - Trigger, which is the product for which registration is sought.

Procedure:
Preliminary Irritation Testing: A group of animals was used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The fur was removed by clipping the dorsal area and flanks of each guinea pig. This area was divided into four test sites (two sites on each side of the midline) on each animal. The test substance was applied neat (100%) and also diluted with distilled water to yield w/w concentrations of 75%, 50%, and 25%. Each concentration was applied (0.4 mL) to a test site using an occlusive 25 mm Hill Top Chamber. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 hours of exposure, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 hours after application, each site was evaluated for local reactions (erythema) according to a scoring system provided in the laboratory report.

From these results, the HNIC (the highest concentration that produced responses in 4 guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for challenge. The HNIC selected for the challenge phase was 100%.

Preparation and Selection of Animals: On the day before initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy, naïve animals (not previously tested) without pre-existing skin irritation were selected for test. Animals were re-clipped prior to each dose.

Induction Phase: Once each week for three weeks, four-tenths of a milliliter of the undiluted test substance was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system.

Challenge Phase: Twenty seven days after the first induction dose, four tenths of a milliliter of the test substance (100%, HNIC) was applied to a naïve site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the challenge application according to the scoring system. In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical environmental conditions and were treated with the HNIC of the test substance at challenge only. These animals constituted the "naïve control" group.

Historical Positive Control: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent validation, EPSL Study #28478, was performed by Eurofins PSL. Testing was completed on December 30, 2009. This test was conducted at the Dayton Facility with Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described above.

Results:

Induction Phase:

Test Animals (100% test substance): Very faint erythema (0.5) was noted for most test sites during the induction phase. [Five of twenty animals showed no irritation.]

Historical Positive Control Animals (100% HCA): Very faint erythema (0.5) was noted for one positive control site following the second induction application. Very faint to faint erythema (0.5-1) was noted for all positive control sites after the third induction.

Challenge Phase:

Test Animals (100% test substance): Very faint erythema (0.5) was noted for six of twenty test sites 24 hours after challenge. Similar irritation persisted at two of these sites through 48 hours.

Naïve Control Animals (100% test substance): Very faint erythema (0.5) was noted for two of ten naïve control sites 24 hours after challenge. Irritation cleared from these sites by 48 hours.

Historical Positive Control Animals (100% HCA): Four of ten positive control animals exhibited signs of a sensitization response (faint erythema [1]) 24 hours after challenge. Similar indications persisted at two of these sites through 48 hours. Very faint erythema (0.5) was noted for all other sites following challenge.

Historical Naïve Control Animals (100% HCA): Very faint erythema (0.5) was noted for one of five naïve control sites 24 and 48 hours after challenge.

Sensitization Response Indices (Erythema)

	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals – Challenge	0 / 20	0 / 20	0.15	0.05
Naïve Control Animals – Challenge	0 / 10	0 / 10	0.10	0.00

[1]Animals with scores greater than 0.5
[2]Sum of the erythema scores divided by the number of animals evaluated

Test Animal Group Skin Reaction Scores

Treatment Phase	Induction						Challenge	
	1		2		3			
Concentration	100%		100%		100%		100%	
Hours[1]	24	48	24	48	24	48	24	48
Animal No. / Sex								
Test Group								
3601 / M	0	0	0	0	0	0	0	0
3602 / M	0.5	0	0	0	0.5	0.5	0	0
3603 / M	0	0	0	0	0	0	0	0
3604 / M	0	0	0.5	0.5	0.5	0.5	0	0
3605 / M	0	0	0	0	0	0	0	0
3606 / M	0	0	0	0	0.5	0	0	0
3607 / M	0	0	0	0	0	0	0.5	0.5
3608 / M	0.5	0	0	0	0	0	0.5	0.5
3609 / M	0	0	0.5	0	0.5	0.5	0	0
3610 / M	0	0	0	0	0.5	0.5	0.5	0
3611 / M	0.5	0	0.5	0.5	0.5	0.5	0.5	0
3612 / M	0.5	0	0.5	0.5	0.5	0.5	0.5	0
3613 / M	0	0	0.5	0	0.5	0	0	0
3614 / M	0.5	0	0.5	0	0.5	0.5	0	0
3615 / M	0	0	0	0	0.5	0	0.5	0
3616 / M	0	0	0	0	0	0	0	0
3617 / M	0.5	0	0	0	0.5	0	0	0
3618 / M	0.5	0	0	0	0.5	0	0	0
3619 / M	0	0	0.5	0.5	0.5	0.5	0	0
3620 / M	0.5	0	0	0	0	0	0	0
Naïve Control Group								
3621 / M	--	--	--	--	--	--	0	0
3622 / M	--	--	--	--	--	--	0	0
3623 / M	--	--	--	--	--	--	0	0
3624 / M	--	--	--	--	--	--	0.5	0
3625 / M	--	--	--	--	--	--	0.5	0
3626 / M	--	--	--	--	--	--	0	0
3627 / M	--	--	--	--	--	--	0	0
3628 / M	--	--	--	--	--	--	0	0
3629 / M	--	--	--	--	--	--	0	0
3630 / M	--	--	--	--	--	--	0	0

[1]Hours after induction or challenge dose